GENETIC INFLUENCE ON PHOTOTAXIS IN DROSOPHILA MELANOGASTER

NORTIN M. HADLER

Department of Biology, Yale University, New Haven, Connecticut

Historically, the behavior of many taxonomic groups of organisms has been treated as invariant. That individual differences were present was realized, but these variations were often dismissed as insignificant deviations from the behavior norm characteristic of the particular organism. Recent research, such as that of Hirsch (1959), Lewontin (1959), Erlenmeyer-Kimling and Hirsch (1961) and others, has demonstrated the inadequacy of this viewpoint. A more complete understanding of the behaving organism requires knowledge of individual differences in the population.

Drosophila melanogaster was the organism chosen by earlier workers for investigation of individual differences and analysis of the genetic contribution to observed behavior. This organism is readily available, has a short generation time, and is quite amenable to genetic analyses. Furthermore, strong geo- and phototaxes can be readily elicited for behavioral and genetic analysis. Hirsch and Tyron (1956) described a reliable technique for assessing the geotactic response of large numbers of individuals of *Drosophila melanogaster*. Hirsch and Boudreau (1958) later applied this technique in studying the heritability of phototaxis in *Drosophila melanogaster*. In this experiment a population of *Drosophila melanogaster* was screened by exposing each individual to a light-dark choice as it passed through a Y-tube. Each individual was tested ten times. Selection pressure was applied over 29 generations through assortative mating to produce strains varying greatly in their characteristic degree of positive phototaxis.

In the present paper a Y-maze for the study of phototaxis in *Drosophila* is described, with which large numbers of flies can be scored with high reliability. Animals passing through this apparatus make 15 successive light/dark choices, and their point of emergence is a measure of the strength of their phototactic response. In addition, two selection experiments are described, and their implications for the problem of phototaxis in *Drosophila melanogaster* and of the analysis of behavior in *Drosophila* in general are discussed.

Method

Apparatus

In 1959 Hirsch described a "multiple unit classification maze" for the mass screening of *Drosophila melanogaster* for geotaxis. I have constructed and used for two years analogous mazes for the study of phototaxis in *Drosophila melanogaster*. The photomaze consists of 15 consecutive Y-units. A population of 200 females and 200 males is introduced into the stem of the first Y, and in passing through

the maze each individual makes 15 consecutive light/dark choices. The animals emerge in 16 collecting tubes, each containing a plug of culture medium. The collecting tube into which a fly emerges establishes how many light or dark choices it has made in passing through the maze. A "Plexiglas" cone (Hirsch, 1959) is inserted in each arm of a Y-unit to minimize re-tracing. A maze of N units has $N(N+1)/2$ Y-units, $N(N+1)$ cones and $(N+1)$ collecting tubes.

The structural unit of the maze is a black nylon (rubber in the case of Maze I) hexagon, $\frac{3}{4}''$ on a side and $\frac{5}{16}''$ in thickness. These are glued, sides parallel, onto a sheet of black "Plexiglas" so as to create alleys $\frac{5}{16}''$ wide and form a pattern of Y-units (Fig. 1). The cones are glued into position in the arms of each Y—a black

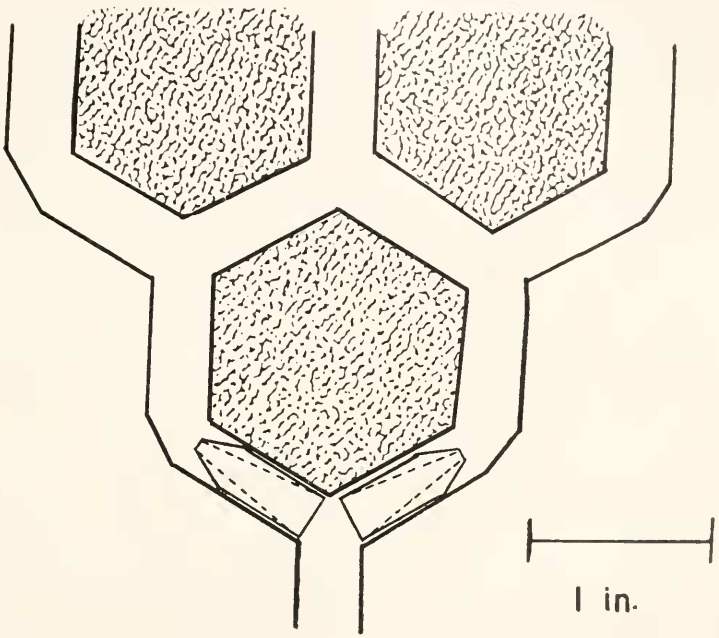

FIGURE 1. Diagramatic representation of the hexagonal structural unit of the photomazes. Alleys are formed by glueing black nylon hexagons onto a sheet of lucite. Lucite cones are inserted in each arm of a Y as shown.

"Plexiglas" cone in each "dark" arm and a clear cone in each "light" arm. A sheet of $\frac{1}{8}''$ "Plexiglas" is fastened over the hexagons by screws, forming a roof over the alleys. The sheet is painted black except over the "light" arm of each Y. The maze is screwed to a blackened sheet of plywood for support, and a circular fluorescent lighting fixture, 12″ in diameter, is suspended 26″ above the horizontal surface. Caution must be exercised in painting and glueing so as not to create a bias of odor or surface texture to compete with the light source as the differential stimulus. For additional information see Hirsch (1959).

Through qualitative observation it appears that mechanical stimulation, rearing conditions, age, temperature and humidity, light, and the effects of gravity must be maintained constant. To control mechanical stimulation while introducing the

TABLE I

Selection data

	Maze I				Maze II			
	Light		Dark		Light		Dark	
G1	♀	♂	♀	♂	♀	♂	♀	♂
$\bar{x}$	6.58	6.84	7.62	8.42	7.92	7.83	8.18	8.99
σ^2	5.35	7.79	4.95	4.94	7.83	7.94	6.16	7.73
G2								
$\bar{x}$	6.56	7.45	8.62	9.51	7.60	8.53	9.57	10.34
σ^2	4.04	5.37	4.88	5.21	6.91	7.79	5.67	7.32
G3								
$\bar{x}$	5.75	5.48	8.53	9.32	6.73	7.75	10.30	10.15
σ^2	7.03	5.37	6.09	6.00	7.26	6.68	4.85	5.54
G4								
$\bar{x}$	6.14	6.92	9.15	9.49	7.56	8.68	10.45	11.49
σ^2	5.28	5.14	6.82	7.85	7.26	7.76	6.36	5.46
G5								
$\bar{x}$	5.34	6.30	8.48	9.84	7.02	8.29	10.54	11.29
σ^2	11.06	8.14	13.19	7.04	8.73	6.80	8.45	6.11
G6								
$\bar{x}$	5.66	4.75	8.77	8.59	5.24	6.02	9.19	9.33
σ^2	5.42	5.54	6.06	9.26	6.14	7.08	4.54	7.60
G7								
$\bar{x}$	4.88	4.49	10.02	10.06	4.70	5.66	8.85	9.66
σ^2	5.10	7.01	5.63	9.94	5.13	5.90	6.16	7.87
G8								
$\bar{x}$	4.82	5.00	10.10	10.07	6.21	6.62	9.66	10.17
σ^2	5.42	7.22	7.03	7.59	7.40	8.26	6.98	6.30
G9								
$\bar{x}$	3.73	3.42	10.65	10.86	4.35	4.68	9.33	9.25
σ^2	4.44	4.87	4.89	6.59	4.07	4.76	6.65	7.72
G10								
$\bar{x}$	4.40	3.96	10.48	10.15	4.12	4.36	8.78	9.88
σ^2	6.45	4.87	5.41	6.29	5.22	4.97	8.38	7.38
G11								
$\bar{x}$	5.62	5.26	12.27	12.52	5.61	6.67	11.22	12.47
σ^2	5.11	5.27	4.08	5.17	5.92	5.38	5.20	5.51
G12								
$\bar{x}$	4.31	4.09	11.24	11.88	4.86	5.30	10.77	11.11
σ^2	5.08	4.59	6.63	4.58	5.73	6.31	4.70	6.76
G13								
$\bar{x}$	3.25	3.72	11.29	12.20	5.13	6.41	11.69	11.64
σ^2	5.49	4.47	5.02	4.05	8.50	8.49	7.93	5.88
G14								
$\bar{x}$	3.52	3.62	11.81	12.76	4.29	4.66	9.72	10.37
σ^2	3.83	4.86	4.13	2.57	6.27	5.54	7.25	6.44
G15								
$\bar{x}$	3.90	4.00	12.60	12.71	3.73	3.79	11.62	12.01
σ^2	3.99	5.47	4.70	4.54	5.10	5.33	5.75	4.45

Wildtype Controls

	Maze I		Maze II	
	♀	♂	♀	♂
$\bar{x}$	7.88 ± .58	8.70 ± .63	9.97 ± .52	10.46 ± .48
σ^2	6.03 ± 1.31	6.55 ± .99	7.72 ± 1.53	6.63 ± .54

animals into the apparatus, a sliding door blocks immediate access to the maze when the starting tube containing the flies is first attached. After sufficient time for the effects of mechanical stimulation, which accompany transfer to the maze, to abate (usually 30 minutes), the door to the maze is opened carefully to avoid agitation.

Two mazes have been constructed in this design, and separate selection experiments are being done with each maze. To increase the differences between the mazes, a double circular fluorescent fixture illuminates the surface of Maze II at about 300 apparent foot candles. Over Maze I, a single bulb fixture is suspended. In both mazes the animals are scored 0–15, corresponding to the numbers of the collecting tubes. Tube 0 receives those subjects which have made 15 consecutive light choices, *i.e.*, from whom the extreme measurable photopositive response has been elicited. Similarly, Tube 15 receives the most photonegative flies.

Subjects and procedure

The selection experiments described in this paper involved over 20,000 flies. The foundation population from which both dark and both light strains were derived was established in a population cage from equal numbers of Formosa, Capetown and Syosset strains of *Drosophila melanogaster* provided through the generosity of Prof. Th. Dobzhansky. Flies from this wild type population were passed through the maze, and selection was begun by mating 60 females and 60 males from the photopositive end of the distribution. Similar matings were done with flies at the photonegative end of the distribution. By this procedure photopositive and photonegative strains were established for each maze. In succeeding generations the extreme 60 males and 60 females from each strain were mated. Maze trials were 24 hours in duration, each beginning at approximately 6 PM to control for diurnal rhythms in behavior. The age of the 200 males and 200 females (run simultaneously) at the time of testing did not exceed 96 hours. Cultures were maintained at room temperature and humidity. The culture medium used in the Yale Laboratories is prepared with the following ratio of ingredients: 56.5 cc. H_2O/0.5 g. agar/6 cc. molasses/4.9 cc. cornmeal/0.7 g. brewers yeast/0.75 cc. 10% tegosept solution.

Results

For fifteen generations selection pressure has been applied to produce highly photopositive ("light") and photonegative ("dark") strains. The results of these trials are given in Table I. Figure 2 shows the phototactic response of each strain as a function of generation number.

Included in Table I are the results of 9 wild type control populations tested with Maze I and 9 tested with Maze II. Presented are the averages of the means of these trials, averages of the variances and their respective standard errors. The number of flies in each trial was approximately constant. Note that because of a difference in stimulus environment, the flies of Maze II were characteristically more photonegative than those of Maze I. Even more interesting is the fact that the variances of the female populations tested in the two mazes are significantly different. However, when a single population was subjected to two consecutive trials in the same maze, the differences in variances were not significant (Table II).

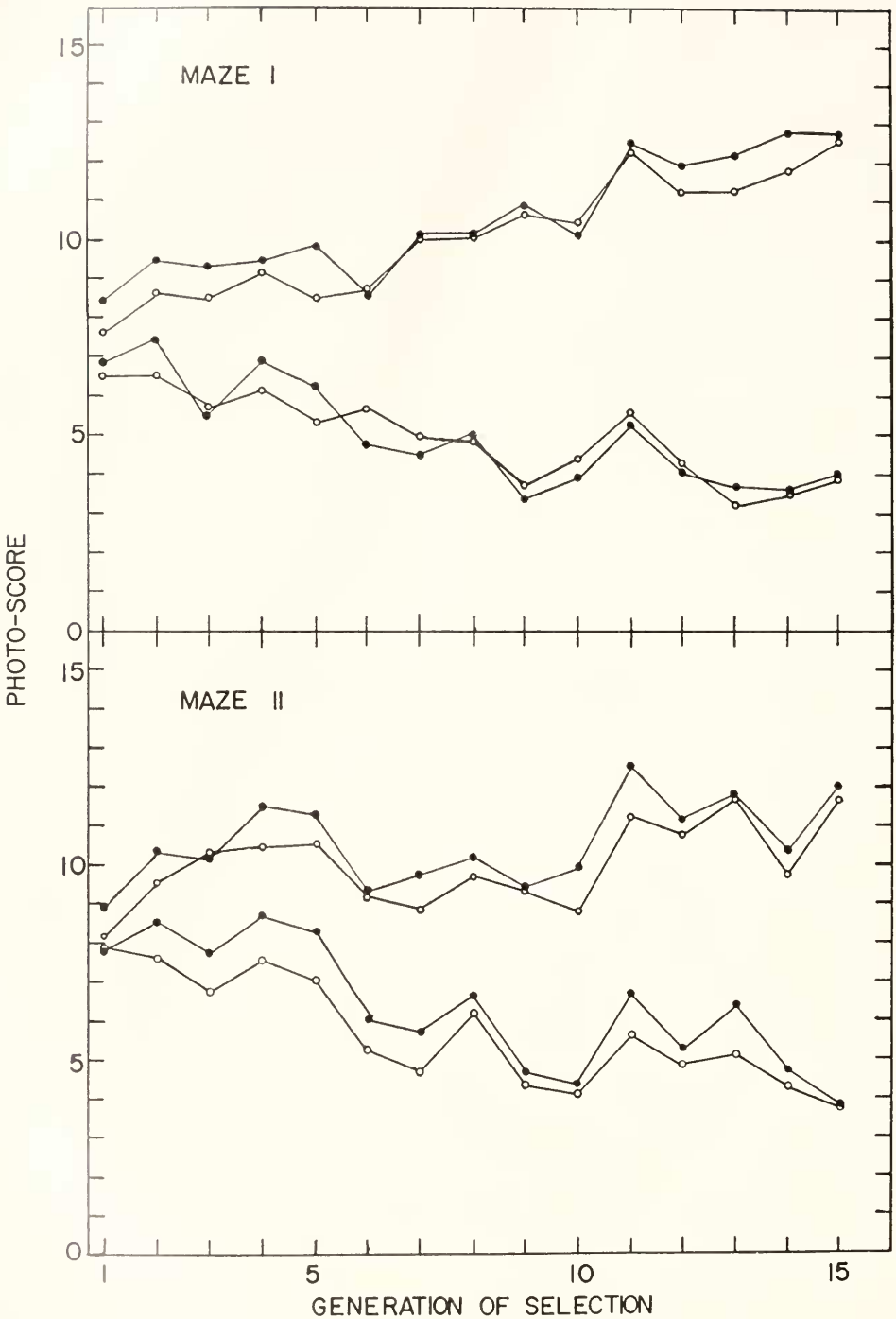
MAZE I
MAZE II
PHOTO-SCORE
GENERATION OF SELECTION
0
5
10
15
1
5
10
15

FIGURE 2.

TABLE II

Rerun data on wild type populations

			n_1	n_2	F	$Pr[t > \|t\|]$
A.	MI	♀	159	151	1.00	0.871
		♂	164	142	1.22	0.997
	MII	♀	190	171	1.02	0.997
		♂	180	180	1.15	0.936
B.	MI	♀	187	35	1.08	0.887
		♂	168	22	1.38	0.781
	MII	♀	209	51	1.24	0.997
		♂	221	43	1.19	0.875

A—rerun of entire population.
B—rerun of modal collecting tube population.
n_1—degrees of freedom of greater mean square.
n_2—degrees of freedom of lesser mean square.
N.B. all F values accept at the 5% level.

This suggests that Maze II elicits a greater variation in behavior in females than does Maze I and that this variation is purely a function of a genotype-environment interaction. After 15 generations of selection, however, neither the means nor the variances in the female population in Maze I were significantly different from those in Maze II. Thus, selection has effected a change in the genetic constitutions of the Maze I and Maze II populations so that the two mazes no longer represent significantly different stimulus environments. A difference in the variances of the flies tested in Maze I and Maze II was not observed in the male populations.

When the variance of the selected population approaches the standard error of the variance of the wild type population, selection will reach a limit. Thus the variances of the selected populations will approach the following asymptotes: Maze I Light Strain female 1.31, Maze I Light Strain male 0.99, Maze II Light Strain female 1.53, Maze II Light Strain male 0.54, and 15 minus these values for the corresponding dark lines. Until these asymptotes are closely approached no reliable estimate of heritability can be made.

From Figure 2 it is obvious that response to selection was immediate and quite strong. The variances of the later selected generations are highly significantly different from those of the unselected foundation populations. This is experimental evidence for the change in the genetic constitution of the population effected by selection in the photomazes. However, rather than compare the means of the selected and unselected populations, information as to the strength of selection can

FIGURE 2. Selection in Maze I and Maze II over 15 generations. The mean of the distribution of each sex of each strain is plotted as a function of generation of selection. The units of "photoscore" correspond to the numbers of the collecting tubes where the flies leave the maze. Flies emerging into Tube 0 have completed 15 consecutive light choices; into Tube 1, 14 light choices and one dark choice. Therefore, flies emerging into Tube 15 have completed 15 consecutive dark choices. Open circles represent females; closed circles, males.

TABLE III

t Tests with wild type and theoretical extreme means

Generation and electrode	Sex	Strain	t	$Pr[t > t_0]$	d	Hypothesis
G13	♀	$M_{II}Lt$	1.39	0.168	138	0
			−1.98	0.049	138	7.90*
	♂		1.76	0.081	133	0
			−2.35	0.020	133	8.70*
	♀	$M_{II}Dk$	−1.65	0.100	189	15.00
			1.52	0.131	189	7.90
	♂		−1.39	0.165	203	15.00
			1.74	0.084	203	8.70
	♀	$M_{III}Lt$	1.76	0.080	166	0
			−1.67	0.097	166	10.00
	♂		2.20	0.029	160	0*
			−1.40	0.162	160	10.50
	♀	$M_{III}Dk$	−1.18	0.242	135	15.00
			0.60	0.550	135	10.00
	♂		−1.39	0.168	147	15.00
			0.47	0.639	147	10.50
G14	♀	$M_{II}Lt$	1.80	0.074	195	0
			−2.24	0.026	195	7.90*
	♂		1.64	0.103	177	0
			−2.30	0.022	177	8.70*
	♀	$M_{II}Dk$	−1.57	0.118	193	15.00
			1.92	0.056	193	7.90
	♂		−1.40	0.164	173	15.00
			2.53	0.012	173	8.70*
	♀	$M_{III}Lt$	1.71	0.088	236	0
			−2.28	0.023	236	10.00*
	♂		1.98	0.049	199	0*
			−2.48	0.014	199	10.50*
	♀	$M_{III}Dk$	−1.96	0.051	254	15.00
			0.10	0.917	254	10.00
	♂		−1.83	0.069	198	15.00
			−0.05	0.958	198	10.50
G15	♀	$M_{II}Lt$	1.95	0.052	164	0
			−2.00	0.047	164	7.90*
	♂		1.71	0.089	168	0
			−2.01	0.046	168	8.70*
	♀	$M_{II}Dk$	−1.11	0.270	203	15.00
			2.17	0.031	203	7.90
	♂		−1.08	0.284	171	15.00
			1.88	0.062	171	8.70
	♀	$M_{III}Lt$	1.65	0.100	214	0
			−2.78	0.006	214	10.00*
	♂		1.64	0.102	196	0
			−2.91	0.004	196	10.50*
	♀	$M_{III}Dk$	−1.41	0.161	201	15.00
			6.68	0.499	201	10.00
	♂		−1.41	0.158	190	15.00
			0.72	0.475	190	10.50

N.B. "d" is number of degrees of freedom, *i.e.*, number of individuals in population−1.
*t Test rejects at 5% level.

be obtained by comparing the means of the selected population with model populations bearing either a mean of 0, 15, or the mean characteristic of the wild type population for a particular maze and sex (from Table I). The results of these *t* tests for the later generations of selection are presented in Table III. In the G15 populations the following lines have diverged significantly from the appropriate wild type mean and are not significantly different from the appropriate extreme mean:

Maze I, "light" males and females
Maze I, "dark" females
Maze II, "light" males and females

The response to selection was apparently less strong for the dark lines than for the light lines. This can be accounted for in part by the fact that the wild type means, especially for Maze II, were in the photonegative half of the photoscoring range, *i.e.*, 7.5. There was therefore less room for screening and selection to operate in the dark side of the photomazes.

Discussion

"Taxis" is defined as "locomotory movement of an organism . . ., in response to a directional stimulus, the direction of movement being oriented in relation to the stimulus" (Abercrombie *et al.*, 1962). The crucial word is "oriented."

Three different experimental designs have been utilized in studying phototaxis in *Drosophila melanogaster:* (1) The rate at which flies approach a light source at the far end of a tube is measured (Carpenter, 1905; Payne, 1911; McEwen, 1918; Scott, 1937, 1943). (2) The distribution of flies in a field with a directed light source is recorded after a specified period (Carpenter, 1905; Lutz and Grisewood, 1934; Fardon *et al.*, 1937; Barigozzi and Tonissi, 1946; Dürrwachter, 1957; Wolken *et al.*, 1957). (3) The flies pass through a Y-tube and the number of animals entering each arm is determined (Brown and Hall, 1936; Fingerman, 1952; Hirsch and Boudreau, 1958). Although the term phototaxis has been used in describing all three of these experimental designs, it is quite obvious these procedures do not measure the same response. The first method confounds phototaxis with photokinesis. That there is a difference between methods (2) and (3) may be less obvious, but it is nevertheless quite real. For example, McEwen (1918) by measuring the spatial distribution in response to directed light source found the tan mutant of *Drosophila melanogaster* to be "negatively phototactic." When screened through my photomazes, a population of tan mutant has a mean performance characteristically more photopositive than that of wild type. However, it must be kept in mind that it is likely that both culture conditions and, even more important, the genetic backgrounds of the mutant stocks differed between the present work and McEwen's. These factors could greatly influence the observed behavior. These remarks indicate one of the major difficulties in comparing results from different laboratories—confusion as to what kind of experimental apparatus is needed to measure phototaxis.

In addition to experimental design there are other factors which make difficult direct comparisons of published data. A review of the literature, coupled with personal observations, indicates some fourteen environmental or experimental variables

that will affect, to some degree, the phototactic response of *Drosophila:* genetic background of the tested population, temperature during the test, time of day of the test, time since anaesthetic, rearing conditions, mechanical stimulation (Lewontin, 1959), time since feeding, energy and wave-length of light (*cf.* Goldsmith, 1961), state of dark adaptation, number of observations or trials per individual (Dürrwachter, 1957), age (Dürrwachter, 1957) and sex. A phototactic response is therefore a property of a particular stimulus environment, broadly defined. Only responses obtained in like environments can be compared.

Finally, phototaxis is a population concept. As shown by Hirsch (1959) and confirmed and extended in the present work, part of the variation in response observed with a population of flies is genetic in origin. Work is presently underway to elucidate both the physiological and genetic differences between the photopositive and photonegative strains.

The author gratefully acknowledges his indebtedness to Profs. D. F. Poulson, J. Hirsch and T. H. Goldsmith for valuable consultation and for providing laboratory space and facilities. The invaluable assistance of Steven Weller in computer analysis and statistical analysis is gratefully acknowledged. This work was supported by National Science Foundation Undergraduate Research Participation Awards administered through Yale University.

Summary

1. The design and construction of two multiple Y-unit mazes are described, which will permit the assessment of the mean and variance of phototactic behavior in *Drosophila* populations.

2. Using maze performances as criteria, selection pressure has been applied for 15 generations. By this procedure highly photopositive and photonegative strains have been produced. The strength and limits of selection in the different mazes are established.

3. By an analysis of the behavior of the selected and unselected strains, the interaction of the environmental and genetic influences on phototactic behavior in *Drosophila melanogaster* is demonstrated.

4. The necessity of recognizing individual differences in populations of experimental animals and the importance of a controlled environment in the study of phototaxis are discussed, with particular reference to *Drosophila.*

LITERATURE CITED

Abercrombie, J. B., C. Hickman and M. Johnson, 1962. A Dictionary of Biology. Aldine Pub. Co., Chicago.

Barigozzi, C., and G. Tonissi, 1946. Ricerche preliminari sul fototropismo dei mutanti di *Drosophila melanogaster* Meig. *Commentationes, Pontificia Academia Scientarum,* 10: 431–459.

Brown, F. A., Jr., and B. V. Hall, 1936. The directive influence of light upon *Drosophila melanogaster* Meig and some of its eye mutants. *J. Exp. Zool.,* 74: 205–220.

Carpenter, F., 1905. Reactions of the pomace fly to light, gravity, and mechanical stimulation. *Amer. Nat.,* 39: 157–171.

Dürrwachter, G., 1957. Untersuchungen über Phototaxis und Geotaxis einiger *Drosophila*—Mutanten nach Aufzucht in verschiedenen Lichtbedingungen. *Zeitschr. Tierpsychologie*, **14**: 1–28.

Erlenmeyer-Kimling, L., and J. Hirsch, 1961. Measurement of the relations between chromosomes and behavior. *Science*, **134**: 1068–1069.

Fardon, J. C., M. J. Carroll and W. A. Sullivan, 1937. Reflection spectra of eye pigment in *Drosophila melanogaster* and its mutants. *Stud. Instit. Div. Thomae*, **1**: 197–206.

Fingerman, M. J., 1952. The role of the eye-pigments of *Drosphila melanogaster* in photic orientation. *J. Exp. Zool.*, **120**: 131–164.

Goldsmith, T. H., 1961. The color vision of insects. *In:* Light and Life, W. D. McElroy and B. Glass, eds., The Johns Hopkins Press, Baltimore, pp. 771–794.

Hirsch, J., 1959. Studies in experimental behavior genetics: II. Individual differences in geotaxis as a function of chromosome variations in synthesized *Drosphila* populations. *J. Comp. Physiol. Psychol.*, **52**: 304–308.

Hirsch, J., and J. C. Boudreau, 1958. Studies in experimental behavior genetics: I. The heritability of phototaxis in a population of *Drosphila melanogaster*. *J. Comp. Physiol. Psychol.*, **51**: 647–651.

Hirsch, J., and R. C. Tryon, 1956. Mass screening and reliable individual measurement in the experimental behavior genetics of lower organisms. *Psychol. Bull.*, **53**: 402–410.

Lewontin, R. C., 1959. On the anomalous response of *Drosophila pseudoobscura* to light. *Amer. Nat.*, **93**: 321–328.

Lutz, F. E., and E. N. Grisewood, 1934. Reactions of *Drosophila* to 2537 Å radiation. *Amer. Mus. Novit.*, **706**: 1–7.

McEwen, R. S., 1918. The reactions to light and to gravity in *Drosophila* and its mutants. *J. Exp. Zool.*, **25**: 49–106.

Payne, F., 1911. *Drosophila ampelophila* Loew bred in the dark for 69 generations. *Biol. Bull.*, **21**: 297–301.

Scott, J. P., 1937. Inherited behavior in *Drosophila*. *Proc. Indiana Acad. Sci.*, **46**: 211–216.

Scott, J. P., 1943. Effects of single genes on the behavior of *Drosophila*. *Amer. Nat.*, **77**: 184–190.

Wolkin, J., A. Mellon and G. Contis, 1957. Photoreceptor structures, II. *Drosophila melanogaster*. *J. Exp. Zool.*, **134**: 383–410.